with Triton clearly displaces the β/α-ratio towards the high molecular weight lipoproteins (those same lipoproteins which increase in human and experimental atherosclerosis, diphenylilethylacetic acid makes the lipoproteic pattern return to normal, inhibiting the formation of β-lipoproteins.

Number of rats	Treatment: Triton mg/kg intravenous	Diphenylilethylacetate* mg/kg intraperitoneal**	Serum cholesterol mg/100 ml (and standard deviation)
50	–	–	77 ± 3·70
50	200	–	269 ± 8·54
15	200	100	152 ± 16·53

* Triton and Sodium diphenylilethylacetate were given at the same time to animals fasted for 12 h. The animals were killed 18 h after administering Triton.

** It has been demonstrated in other experiments that diphenylilethylacetic acid can inhibit the effects of Triton also when given *per os* (250 mg/kg). Diphenylilethylacetic acid does not cause variations of normal cholesteremia when used in the doses reported by us.

Number of rats	Triton mg/kg	Treatment: Diphenylilethylacetate mg/kg intraperitoneal *	Serum total lipids mg/100 ml	Lipoproteins**			
				α	β	X	β/α
30	–	–	200 ± 15·93	34·8	48·4	14·8	1·3
15	200	–	1008 ± 56·24	11·52	71·7	16·2	6·2
10	200	100	481 ± 49·37	23·15	53·8	22·6	2·2

* We demonstrated in other experiments that diphenylilethylacetic acid can inhibit the effects of Triton also when given *per os* (250 mg/kg).

** In the present elaboration of data, lipoproteins have been divided on the grounds of their localization on the protein electrophoretic pattern.

Diphenylilethylacetic acid, which is very well tolerated by man in doses of 300 mg daily, has been used recently also in clinical trials and gave good results in cases of hypercholesteremia[13].

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Résumé

On prouve que l'acide diphénylilléthylacétique, qui exerce un effet d'inhibition sur l'activité acétylante du coenzyme A, peut aussi causer une diminution de l'hypercholestérolémie et de l'hyperlipémie provoquées par une administration de Triton.

[13] G. ANNONI, Farmaco *11*, 244 (1956).

Isolation of a Cytochrome of the Antimycin A Sensitive Pathway for DPNH Oxidation

A cytochrome has been isolated from pig, guinea pig or rat liver, having the following absorption peaks: 404 and 500 mμ in oxidized state and 428 and 556 mμ in reduced state.

This cytochrome was prepared from liver using essentially the method of EDELHOCH *et al.*[1] for DPNH-cytochrome c reductase. With rat and guinea pig liver the cytochrome is not extracted by 10% ethanol at 40° C, but can be obtained by a subsequent extraction with *M*/20 orthophosphate buffer pH 7·4. With pig liver this cytochrome is obtained in the ethanol extract, together with DPNH-cytochrome c reductase, but can be separated from it by ammonium sulfate fractionation.

This cytochrome is reduced by hydrosulfite, and leucomethylene blue, but not by reduced glutathione or ascorbic acid. It is not reduced by DPNH-cytochrome c reductase, unless SLATER[2] factor is present.

To obtain the Slater factor, rat liver homogenate is taken to pH 5·4, and the precipitate extracted with a digitonin solution. Reduction of the present cytochrome by DPNH-cytochrome c reductase is a specific test for this factor, the reaction being inhibited by antimycin A. In this reaction Slater factor can be replaced by methylene blue, which is reduced by the reductase and reoxidized by the present cytochrome, but not inhibited by antimycin A.

It is remarkable that DPNH-cytochrome c reductase is able to reduce cytochrome c directly, but the present cytochrome, which has a lower redox potential, only through Slater factor.

0·01 *M* orthophosphate inhibits the reduction of cytochrome c, by reductase, probably by combining with its iron[3], and inhibits also the reduction of the present cytochrome. Removal of the bound iron of the reductase by dialysis against 8-hydroxyquinoline transforms this enzyme to a diaphorase, which reduces the new cytochrome even in the presence of orthophosphate.

Although the present cytochrome has a different absorption spectrum from the other cytochromes recently described[4], it may turn out to be identical with one of them.

The present cytochrome transports electrons from leucomethylene blue to hydroxylamine. This fact has been erroneously interpreted[5], as hydroxylamine inhibiting the reduction of methylene blue by reductase. This "inhibition" required a factor which had been called hydroxylamine inhibition factor. The factor is now identified with the present cytochrome, which reoxidizes the methylene blue, reduced by the reductase. It was shown[6] that this cytochrome is necessary for the oxydative phosphorylation coupled to DPNH oxidation by cytochrome c, in a soluble system.

I. RAW

Departamento de Química Fisiológica, Faculdade de Medicina da Universidade de Sao Paulo, Brasil, April 17, 1956.

[1] H. EDELHOCH, O. HAYAISHI, and L. J. TEPLY, J. biol. Chem. *197*, 97 (1952).

[2] E. C. SLATER, Biochem. J. *46*, 499 (1950).

[3] H. R. MAHLER and D. G. ELOWE, J. biol. Chem. *210*, 165 (1954).

[4] D. KEILIN and E. F. HARTREE, Nature *176*, 200 (1955). – R. KUNN, Proc. Soc. exper. Biol. Med. *77*, 441 (1955). – C. WILDMER, H. W. CLARK, H. A. NEUFELD, and E. J. STOTZ, J. biol. Chem. *210*, 861 (1954). – R. W. EASTABROOK, Fed. Proc. *14*, 45 (1955).

[5] I. RAW, Science *118*, 159 (1953).

[6] I. RAW, J. Amer. chem. Soc. *77*, 503 (1955).

Zusammenfassung

Ein neues Cytochrom ist aus Schweineleber isoliert worden. Es wird durch DPNH-Cytochrom-c-Reduktase reduziert. Antimycin A hemmt diese Reaktion, die nur in Gegenwart des Slater-Faktors abläuft.

Es ist wahrscheinlich, dass dieses Cytochrom in die Elektronentransportreihe, und zwar zwischen Reduktase und Cytochrom c, eingeschaltet ist.

Effects of Ultraviolet Light on some of the Electrical Characteristics of Action Potentials of Single Unmyelinated Nerve Fibers of the Crab *Carcinus*[1]

As a corollary to photochemical work done in this laboratory with single myelinated nerve fibers[2], a number of experiments have been carried out to test the effects of monochromatic ultraviolet irradiation on resting single unmyelinated fibers from the walking leg of the crab, *Carcinus maenas*.

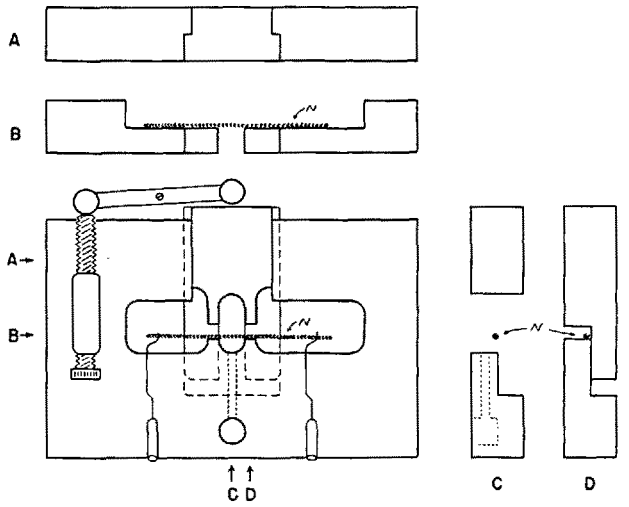

Fig. 1. – Top view and cross-sections of bridge used for irradiation of single crab neurons. Further description in text.

The nerve was obtained by the "pullout" technique of FURUSAWA[3], and a single large fiber (20–30 μ in diameter) was isolated, the dissection being carried out in artificial sea water[4]. After isolation, the nerve was mounted on the bridge diagrammed in Figure 1. This apparatus consisted basically of three pieces of perspex, one a large block, not shown in the Figure, which served as a base for the bridge and which held a quartz window directly under the region of the nerve to be irradiated. The upper part of this bridge, shown in the drawing, was screwed onto the base. It was so constructed that the fiber (*N*) lay across three wells containing sea water. The ends of the fiber were clamped to the floors of their respective wells by stainless steel electrodes. The center well was connected by a tunnel to a fourth well, in which was immersed a Ag–AgCl electrode in sea water agar. The channel in which the nerve lay could be altered in width by a screw-and-lever mechanism which allowed fine movement of a separate, close-fitting, perspex block from which the walls of one side of the channel had been machined. With the nerve in position, the width of these channels could be brought to within a few microns of the diameter of the fiber, providing a high electrical resistance between the three wells without mechanical damage to the fiber itself. It will be seen from cross sections *B* and *C* that the space under the nerve in the center well was open, that is, filled only with sea water. The floor of this space was formed by the quartz window in the base block, through which the entire length of the fiber in the center well (0·6 mm across) could be irradiated from underneath.

The circuit for stimulating and recording is that shown in Figure 2. A Grass stimulator provided monophasic square wave impulses of 0·5 ms duration at a frequency of 10/s. The intensity of the shock could be read, in relative values, from a graduated potentiometer. For protection of the nerve, a resistance of 470 kΩ and capacity of 0·01 μf were placed in series across the stimulating electrodes. Another capacity and resistance, 200 kΩ and 100 pf, (RC = 20 μs) could be switched into the recording circuit for obtaining "differentiated action potentials" on the oscillograph. Variables measured during any given experiment were threshold, height of the spike, and, from the "differentiated action potential", the rate of rise of the spike.

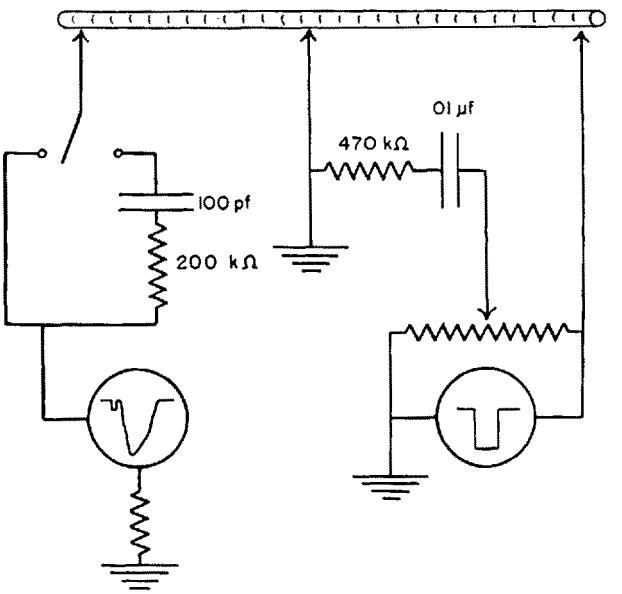

Fig. 2. – Diagram of stimulating and recording circuit. Square waves from Grass stimulator (right); recordings over de Gruyter pre-amplifier and Dumont double-beam cathode ray oscillograph (left).

Radiation of $\lambda = 265$ mμ was obtained by double monochromation of radiation from a Philips high pressure mercury arc. The monochromator used has been

[1] This work was carried out during the tenure of a Postdoctoral fellowship from the United States Public Health Service.

[2] M. HUTTON-RUDOLPH, Helv. physiol. Acta *1*, C15 (1943); Dissertation, Bern 1944. – J. BOOTH, A. v. MURALT, and R. STÄMPFLI, Helv. physiol. Acta *8*, 110 (1950). – A. v. MURALT and R. STÄMPFLI, Helv. physiol. Acta *11*, 182 (1953). – H.-C. LÜTTGAU, Helv. physiol. Acta *12*, C56 (1954); Pflügers Arch. ges. Physiol. *262*, 244 (1956).

[3] K. FURUSAWA, J. Physiol. *67*, 325 (1929).

[4] Based on an analysis of North Sea water, with the following ionic molarities (m*M*/*L*): Na^+ – 477·4; K^+ – 9·0; Mg^{++} – 55·0; Ca^{++} – 8·1; Cl^- – 552·2; $SO_2^=$ – 28·0; NO_3^- – 1·2; HCO_3^- – 2·6; $HPO_4^=$ – 0·3. Total ionic molarity – 1·1338.